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The Rate of Utilization of Urea, Ammonium, and Nitrate by Natural Populations of Marine Phytoplankton in a Eutrophic Environment[1]

WAYNE A. HARVEY[2] AND JOHN CAPERON[3]

ABSTRACT: The utilization rates of ammonium, nitrate ion, and urea were determined for 18 samples of water from the southern sector of Kaneohe Bay, Oahu, Hawaiian Islands. The samples were collected from 14 May through 23 August 1974. The mean daytime uptake rates for this period were 0.040, 0.033, and 0.013 hr^{-1} for ammonium, urea, and nitrate, respectively. Dark uptake rates for ammonium, urea, and nitrate from two samples were approximately 50, 30, and 0 percent of the daytime uptake rates. The uptake data indicate that the phytoplankton growth rate is not limited by the availability of fixed nitrogen. This conclusion is supported by the data on the carbon:nitrogen ratio of the phytoplankton, which show that the plants were more heavily enriched in nitrogen than they had been during previous studies of this part of the bay. Mass balance calculations show that the supply of fixed nitrogen to the nutrient pool from stream runoff and municipal waste discharge was only 3.5 percent of the total uptake rate by phytoplankton, and, therefore, suggest that the *in situ* regeneration of nutrients is far larger than the new nutrients added to the bay from these sources.

DUE TO THE RELATIVELY HIGH PHYTOPLANKTON REQUIREMENT for fixed nitrogen and to its frequently low concentration in the euphotic zone, the assimilation of inorganic nitrogen has been extensively studied by a number of investigators (Dugdale, Menzel, and Ryther 1961; Dugdale, Goering, and Ryther 1964; Eppley and Coatsworth 1968; MacIsaac and Dugdale 1969; Eppley, Rogers, and McCarthy 1969; Goering, Wallen, and Nauman 1970; Eppley, Rogers, and McCarthy 1971; Caperon and Meyer 1972*a*, *b*). As a result, the uptake kinetics for these chemical species and their role as limiting nutrients for various offshore neritic, oceanic, and laboratory chemostat systems are well documented.

Although inorganic nitrogen has been shown to be an important factor in controlling the production of marine phytoplankton, the role of dissolved organic nitrogen (DON) sources remains largely unknown. Harvey (1940) appears to have been the first investigator to show that certain species of phytoplankton could assimilate urea and uric acid directly, although their growth rates were slower in this medium than in one containing ammonium and nitrate. More recently, Syrett (1962), Guillard (1963) and Carpenter, Remsen, and Watson (1972) have shown that a large number of species isolated from estuarine and nearshore neritic environments are capable of utilizing urea effectively. In the case of the Savannah estuary, Remsen, Carpenter, and Schroeder (1972*a*) found that the phytoplankton population competed with bacteria for DON and, as a result, considered them to be the primary consumers of urea. McCarthy (1972*a*) also demonstrated that several species of marine phytoplankton are capable of utilizing ecologically significant concentrations of urea. Consequently, in addition to nitrate and ammonium, urea must also be considered as part of the nitrogen pool available for growth of phytoplankton. The work reported here represents an attempt to determine the uptake rates of urea, ammonium, and nitrate by natural populations of phytoplankton in Kaneohe Bay.

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[2] University of Hawaii, Department of Oceanography, 2525 Correa Road, Honolulu, Hawaii 96822. Present address: Environmental Dynamics, Inc., 1609 Westwood Boulevard, Suite 202, Los Angeles, California 90024.

[3] University of Hawaii, Department of Oceanography, 2525 Correa Road, Honolulu, Hawaii 96822.

Study Location

Kaneohe Bay (Figure 1) is a subtropical estuary located on the northeastern coast of Oahu, Hawaiian Islands. The bay is approximately 12.8 km long and 4.3 km wide, with its circulation restricted by a large barrier reef that separates most of the bay from the open ocean. The water temperature in the bay has an annual range of 19.5°–27.8° C (Smith, Chave, and Kam 1973). The southern sector of the bay, where the research was conducted, has the most restricted circulation and is the location of the main municipal sewage treatment operation. Caperon, Cattell, and Krasnick (1971) estimated that 9.5×10^6 liters of secondarily treated sewage are added daily by the sewage treatment plant to the southern sector of the bay. Analyses of a single sample of sewage effluent indicated that the input concentrations for urea, ammonium, and nitrate are in the range of, respectively, 23, 1590, and 48 μg-at/liter. Considerable interest exists as to the rate at which these nutrients are withdrawn from the system.

Nutrient Limitation of Phytoplankton Growth

The evidence that nitrogen is the limiting nutrient in the southern sector is not conclusive. The ratio of inorganic nitrogen:phosphorus in input waters is much lower (5.35:1) than the often-quoted 15:1 ratio for deep ocean waters, and nutrient data (unpublished) for Kaneohe Bay indicate that inorganic nitrogen in the water approaches zero in the presence of significant amounts of phosphate and silicate.

Redfield, Ketchum, and Richards (1963) observed that "normal" marine phytoplankton has a C:N ratio (atom:atom) of 6.6:1, whereas nitrogen-deficient cells have a higher ratio, 25.9:1. Caperon and Meyer (1972*a*) noted that the data on several single species of phytoplankton grown in open-ocean seawater enriched with material from the sewage outfall in the bay indicate similar nitrogen-deficient C:N ratios. Thus, prior to urbanization of the watershed area and commencement of municipal waste discharge, the bay was probably limited by the supply of fixed nitrogen and could well return to this state if the discharge of wastes were to cease.

EXPERIMENTAL METHODS

Seawater samples for the uptake experiments were collected from five different stations (Figure 1), over a 3-month period from 14 May through 23 August 1974, in a 5-liter water sampler and were passed through 102-μ nylon mesh into a 20-liter plastic carboy. The stations were selected because they cover an ecologically significant range of *in situ* nutrient concentrations. A sampling depth of 3 meters was chosen because other productivity studies conducted in the same region had indicated that this was the depth of maximum primary production (Lamberson 1974). In addition, the samples were collected before sunrise to assure that the phytoplankton had been preconditioned by uniform light conditions.

Chlorophyll Analyses

Chlorophyll analyses were conducted to determine variations in population size-fractions for each experiment. Samples prefiltered through 102-μ, 35-μ and 20-μ nylon mesh were filtered onto glass filters, grade C, and frozen in 90 percent acetone before being analyzed. The analytical methods described by Strickland and Parsons (1968) were utilized to determine the trichromatic chlorophyll-*a* and phaeophytin concentrations. We calculated the corrected, living-chlorophyll-*a* value by multiplying the trichromatic chlorophyll-*a* concentration by the chlorophyll-*a*:total pigment (chlorophyll-*a*+phaeophytin) ratio (Lorenzen 1967).

Population Biomass

Particulate organic carbon and nitrogen were determined as described by Caperon, Harvey, and Steinhilper (1976), and these data, together with the chlorophyll-*a* results, were used to determine phytoplankton carbon and nitrogen as also described by these workers.

Nutrient Analyses

The method employed for urea analyses was the diacetyl-monoxime procedure of Newell, Morgan, and Cundy (1967). Experimental tests in which serial dilutions of known standards were used indicated that the procedure has an accuracy (mean value $\pm$ 2 S.D.) of .026 $\pm$ .036 percent and a precision (mean value $\pm$ 2 S.D.)

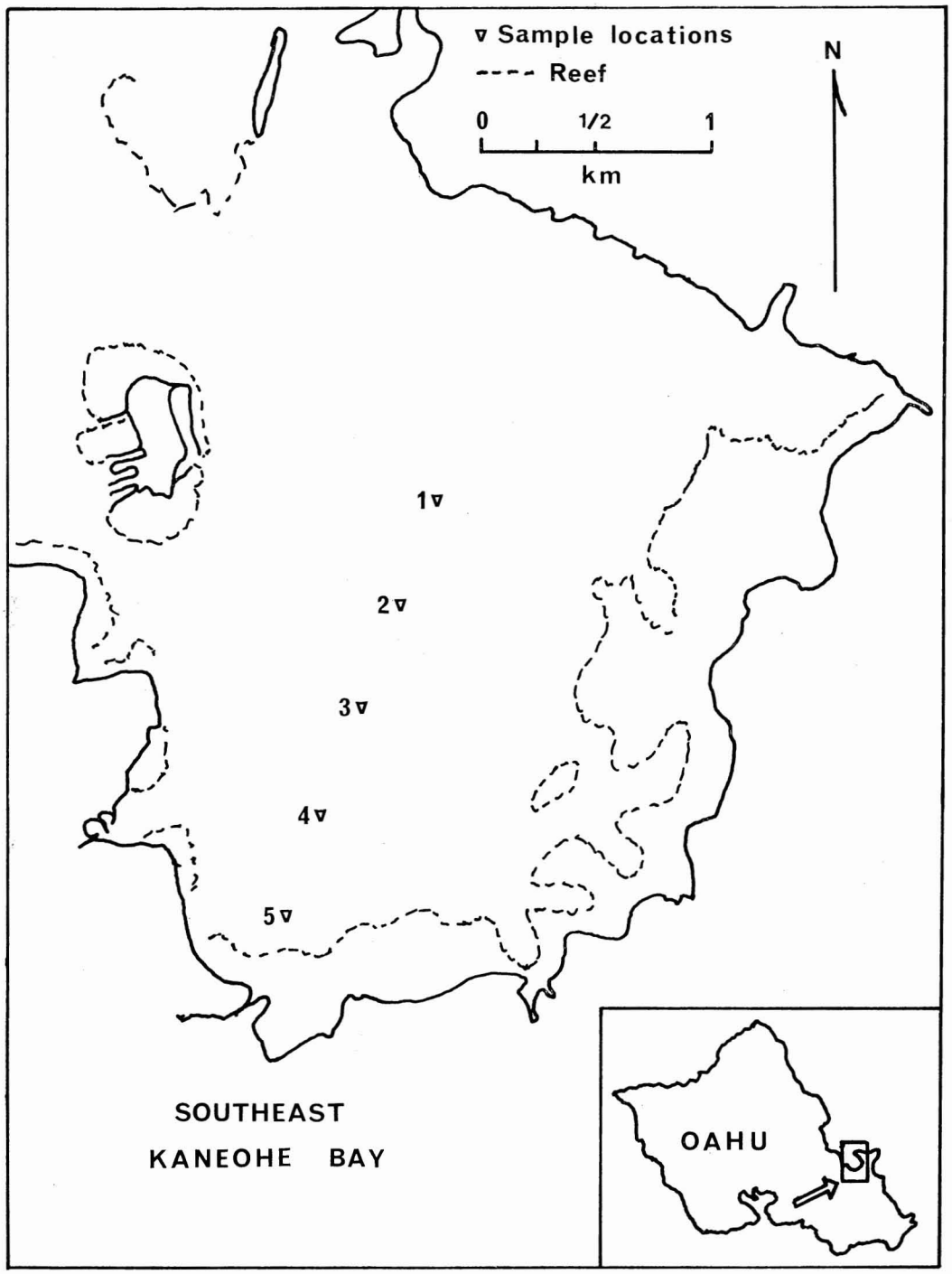

FIGURE 1. Five sampling locations for nutrient uptake experiments conducted in the southern sector of Kaneohe Bay, 14 May–23 August 1974.

of .026 ± .026 per cent. Ammonium and nitrate-nitrite analyses were performed on a Technicon Auto-Analyzer II; the Solarzano (1969) method was utilized for ammonium ions and the cadmium-copper reduction method (Wood, Armstrong, and Richards 1967) for nitrate-nitrite.

^{14}C-Urea Uptake

The ^{14}C-labeled urea method employed by Carpenter, Remsen, and Watson (1972) and Remsen, Carpenter, and Schroeder (1972*a*, *b*) was modified and used to measure the urea uptake rate. Seawater subsamples of 150 ml each were placed into two sets (I, II) of 250-ml Erlenmeyer flasks consisting of light and dark bottles. Set I was used to determine the maximum rate of urea uptake (V_m) while set II was employed to measure the *in situ* urea uptake rate (V). In addition, each set of flasks was partitioned so that the labeled particulate and excreted CO_2 fractions of the utilized urea could be measured. For the first nine experiments, set I was inoculated with a 1-ml urea solution containing 1.2 μg-at unlabeled urea and .164 μCi ^{14}C-urea. Set II was inoculated with .016 μCi ^{14}C-urea, with the exceptions of experiments 17 and 18 which contained .082 μCi ^{14}C-urea. An attempt was made in all cases to keep the labeled urea concentration to < 10 percent of the total urea concentration in set II.

The samples measuring the excreted CO_2 fraction of the urea uptake were sealed with rubber caps, an ' a glass filter was moistened with .15 ml hyamine hydroxide and suspended in a stirrup inside the flask. The samples measuring the labeled particulate organic fraction were also sealed with rubber caps. The flasks were then placed on a shaker table outside the laboratory and an opaque sun screen was set up to protect the samples from direct sunlight. After being incubated for 3 to 4 hours, the particulate samples were filtered on Millipore EH (cellulose-acetate; pore size 0.5 μ) filters. The filters were then rinsed with approximately 50 ml of glass-filtered, unlabeled seawater and placed in scintillation vials with 15 ml of scintillation cocktail, composed of toluene, Triton X-100, and PPO (2,5-diphenyloxazole). The experiments, designed to measure excreted CO_2, were terminated after the same 3–4-hour incubation period by the addition of .3 ml of 50-percent trichloroacetic acid. These flasks were shaken an additional 3–4 hours. The filters were then removed and placed in scintillation vials with 15 ml of scintillation cocktail. In order to reduce the effects of chemiluminescence and suspended O_2 interference, we allowed the vials to sit in the dark for 1 week before counting the radioactivity. The samples were then placed in a Beckman Instruments LS-230 liquid scintillation counter and counted.

We ran sample blanks for each set of flasks simultaneously, using filtered seawater inoculated with the same urea solutions. We determined the efficiency of the CO_2 collection procedure by inoculating filtered seawater samples with ^{14}C-bicarbonate and then treating them as previously outlined for the excreted CO_2 fraction.

Ammonium, Nitrate, and Urea Uptake Experiments

Seawater subsamples of 150 ml each, with no nutrients added, were placed in each of seven flasks, which were then sealed and placed on the shaker table. Time series analyses were conducted over the same 3–4-hour incubation period, with one of the flasks being removed every 30–45 minutes and its contents filtered through a grade C glass filter. The filtrates were then refrigerated and analyzed within 4–6 hours for ammonium, nitrate, and urea. No effects due to refrigeration of the filtrate were observed.

RESULTS AND ANALYSIS

Population Characteristics and Nutrient Concentration

The phytoplankton represented in the size-fraction of < 20 μ (Table 1) contained the predominant portion of the total chlorophyll-*a* concentration. In 12 of 17 experiments, this size-fraction accounted for more than 50 percent of the pigment concentration and averaged 58.5 percent of all samples. The variability within the range, as represented by a relative standard deviation of .309, was the lowest of the three size-fractions observed. The other size-

TABLE 1

SIZE-FRACTION CHLOROPHYLL-*a* CONCENTRATION (μg/liter) AND PERCENTAGE OF TOTAL CHLOROPHYLL-*a* CONCENTRATION, KANEOHE BAY, OAHU, 14 MAY–23 AUGUST 1974

EXPERIMENT NUMBER	TOTAL CHLOROPHYLL-*a*	SIZE 102–35 μ	PERCENTAGE	SIZE 35–20 μ	PERCENTAGE	SIZE < 20 μ	PERCENTAGE
1	3.192	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2	2.049	0.020	00.98	0.829	40.46	1.200	58.57
3	1.197	0.318	26.57	0.194	16.21	0.685	57.23
4	5.361	0.907	16.92	0.776	14.47	3.678	68.61
5	2.877	0.821	28.54	0.743	25.83	1.313	45.64
6	3.328	1.619	56.27	0.369	12.83	1.340	46.58
7	4.786	1.919	40.10	1.663	34.75	1.204	25.16
8	5.582	1.072	19.20	1.322	23.68	3.188	57.11
9	1.399	0.275	19.66	0.746	53.32	0.378	27.02
10	1.482	0.000	00.00	0.314	21.19	1.168	78.81
11	1.247	0.210	16.84	0.335	26.86	0.702	56.30
12	1.388	0.158	11.38	0.252	18.16	0.978	70.46
13	4.163	0.695	16.69	0.352	8.46	3.116	74.85
14	4.619	1.911	41.37	0.470	10.18	2.238	48.45
15	1.596	0.000	00.00	0.000	00.00	1.596	100.00
16	2.253	0.781	34.66	0.000	00.00	1.472	65.34
17	4.218	0.778	18.44	0.895	21.22	2.545	60.34
18	4.298	1.692	39.37	0.261	6.07	2.345	54.56
Mean	3.058	.775	22.76	.560	19.63	1.714	58.53
S.D.	1.532	.671	15.86	.450	14.06	.967	18.08
Relative S.D.	.501	.865	.697	.804	.717	.564	.309

TABLE 2

PARTICULATE ORGANIC CARBON AND NITROGEN, KANEOHE BAY, OAHU, 14 MAY–23 AUGUST 1974

EXPERIMENT NUMBER	CARBON (μg-at/liter)	NITROGEN (μg-at/liter)	CARBON:NITROGEN (atom:atom)
1	9.520	2.552	3.730
2	7.215	.829	8.703
3	2.310	.451	5.122
4	23.254	4.886	4.759
5	16.387	3.601	4.551
6	17.368	3.601	4.823
7	33.456	6.398	5.229
8	40.421	6.876	5.879
9	7.256	1.736	4.180
10	7.582	1.567	4.839
11	10.722	1.819	5.894
12	13.665	2.777	4.921
13	25.240	5.297	4.765
14	33.284	6.002	5.545
15	15.692	2.676	5.864
16	17.916	3.079	5.816
17	22.428	4.322	5.189
18	25.044	4.070	6.153
Mean	18.264	3.474	5.331
S.D.	10.465	1.888	1.060
Relative S.D.	.573	.543	.199

NOTE: Figures adjusted for nonplant particulate fraction.

TABLE 3

AMBIENT NUTRIENT CONCENTRATIONS AND PERCENTAGE OF TOTAL NITROGEN AVAILABLE, KANEOHE BAY, OAHU, 14 MAY–23 AUGUST 1974

EXPERIMENT NUMBER	UREA (μg-at/liter)	% TOTAL NITROGEN AVAILABLE	NH_4^+ (μg-at/liter)	% TOTAL NITROGEN AVAILABLE	NO_3^-–NO_2^- (μg-at/liter)	% TOTAL NITROGEN AVAILABLE
1	.908	54.8	.644	38.9	.104	6.3
2	.466	44.0	.456	43.0	.138	13.0
3	.744	63.1	.168	14.2	.268	22.7
4	2.083	42.0	2.308	46.6	.564	11.4
5	1.884	57.8	1.088	33.4	.288	8.8
6	2.168	31.8	3.060	44.9	1.590	23.3
7	.739	59.7	.412	33.3	.086	7.0
8	1.589	66.0	.620	25.7	.200	8.3
9	1.009	47.5	.497	23.4	.620	29.2
10	1.463	50.2	.820	28.1	.632	21.7
11	1.871	65.6	.884	31.0	.098	3.4
12	2.041	43.3	2.092	44.4	.584	12.4
13	1.555	79.2	.228	11.6	.180	9.2
14	1.109	44.4	1.072	42.9	.316	12.7
15	1.142	74.8	.320	21.0	.064	4.2
16	.958	59.4	.484	30.0	.170	10.5
17	2.779	76.4	.764	21.0	.096	2.6
18	1.097	60.4	.580	31.9	.140	7.7
Mean	1.421	56.7	.917	31.4	.341	11.9
S.D.	.618	13.1	.786	10.6	.369	7.5
Relative S.D.	.435	.231	.857	.339	1.082	.633

fractions, 102–35 μ and 35–20 μ, contained a smaller portion of the total chlorophyll-*a* concentration, with mean values of 22.8 and 19.6 percent, respectively, and exhibited significantly higher variability about the mean, with respective coefficients of variation of .697 and .717. Therefore, the data imply that the principal spatial and temporal variations within the phytoplankton community occur in the 102–35 μ and 35–20 μ ranges and that the $< 20\ \mu$ fraction remains relatively constant in terms of total pigment concentration and percentage of total chlorophyll-*a* present.

The linear regressions of the particulate organic carbon and nitrogen concentrations on total chlorophyll-*a* are both significant at the .1 percent level. The *y*-intercept for the two regressions, therefore, can be interpreted as an estimate for the nonplant fraction of the particulate organic matter. We adjusted the particulate carbon and nitrogen values for the nonplant fraction by subtracting the *y*-intercept obtained in the regressions; these are presented in Table 2. (Refer to Caperon, Harvey, and Steinhilper 1976 for a detailed justification of this procedure.) The linear regression of particulate organic carbon on nitrogen yielded a C:N ratio (atom:atom) of 5.4:1, with a correlation coefficient significant at the .1-percent level.

Ambient nutrient concentrations for each of the experiments are given in Table 3. Mean concentration values (μg-at N/liter $\pm$ 2 S.D.) indicate that urea was generally found in highest abundance (1.421 ± 1.236), ammonium was second highest ($.917 \pm .572$), and nitrate was lowest ($.341 \pm .738$). Urea averaged 56.7 percent and ranged from 31.8 to 79.2 percent of the total nitrogen available. Ammonium and nitrate, on the other hand, accounted for 31.4 and 11.9 percent of the total nitrogen available. In addition to being influenced by phytoplankton uptake, the variability in the mean nutrient concentration was, undoubtedly, affected by a combination of factors including zooplankton excretion, sewage effluent, rainfall, and runoff from the Kaneohe Bay watershed region, which were not monitored during the experimental period.

TABLE 4

NET NUTRIENT UPTAKE RATES DERIVED FROM ^{14}C-UREA EXPERIMENTS AND TIME SERIES NUTRIENT ANALYSES, KANEOHE BAY, OAHU, 14 MAY–23 AUGUST 1974

EXPERIMENT NUMBER	^{14}C-UREA UPTAKE (hr^{-1})		TIME SERIES NUTRIENT ANALYSIS					
			UREA UPTAKE RATE		NH^4 UPTAKE RATE		NO_3 UPTAKE RATE	
	V_m (I)	V (II)	V (hr^{-1})	TOTAL V	V (hr^{-1})	TOTAL V	V (hr^{-1})	% TOTAL V
1	.090	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2	.062	.065	.070	37.9	.106	57.3	.009	4.9
3	.062	.069	.065	40.4	(.015)	–	.081	–
4	.024	.011	.024	16.3	.110	74.7	.013	8.8
5	.029	.021	.028	32.5	.047	54.5	.011	12.8
6	.009	.008	.013	7.5	.142	81.7	.019	10.9
7	.067	.025	.024	85.3	.003	10.7	.001	3.6
8	.026	.024	.035	74.0	.010	21.1	.002	4.2
9	.018	.017	.013	16.5	.030	38.0	.036	45.6
10	.066	.097	.098	55.3	.052	29.3	.027	15.2
11	.019	.013	.057	53.9	.047	44.4	.002	1.9
12	.014	.013	.013	7.2	.160	88.9	.007	3.9
13	.019	.015	.024	70.8	.007	20.6	.003	8.8
14	.025	.014	.027	44.7	.024	39.7	.009	14.9
15	.016	.013	.007	100.0	(.021)	–	.000	0.0
16	.027	.016	.030	88.2	(.018)	–	.004	11.8
17	.026	.028	.030	78.6	.008	21.0	.000	0.0
18	.036	.028	.055	100.0	(.005)	–	(.001)	–
Mean	.035	.028	.035	53.5	.040	44.8	.013	9.8
S.D.	.023	.025	.025	31.5	.057	25.2	.020	
Relative S.D.	.660	.886	.708	.588	1.399	.564	1.543	

NOTE: V_m (I), maximum urea uptake rates from set I experiments; V (II), *in situ* uptake rates from set I. Time series uptake rates have been determined by the linear regression of the nutrient concentration on time. In all cases, the uptake rate, V, represents the rates (μg-at/liter/hr) per unit population nitrogen (μg-at/liter). Rates in parentheses represent net excretion rates.

Uptake of ^{14}C-labeled Urea

The mean uptake rates (± 2 S.D.) for both sets of samples (I, II) were $.035 \pm .046$ hr^{-1} and $.028 \pm .050$ hr^{-1}, respectively (Table 4). In 13 of the 17 experiments, the *in situ* urea uptake rates (V) were not significantly different from the maximum rates (V_m) and suggest that both sets of experiments were measuring the maximum uptake rate. The regression of the maximum uptake rate (V_m), as determined in set I, on the *in situ* uptake rate (V) from set II (Figure 2) has a correlation coefficient that is significant at the .1-percent level. If the extreme values introduced by experiments 7 and 10 are omitted, the t-test for the slope indicates that the regression is not significantly different from the expected 1 at the 5-percent level. In addition, the t-test for the difference between the mean values indicates that there is no significant difference between the two values ($t = .507$). Consequently, it is apparent that both of the uptake experiments were conducted with concentrations of urea at near saturation.

Uptake of Urea Determined by Time Series Nutrient Analysis

The *in situ* urea uptake rates derived from the time series nutrient analyses are given in Table 4. The urea uptake rate accounted for 53.5 percent of the total nitrogen uptake rate and ranged from 7.2 to 100.0 percent. The mean uptake rate (± 2 S.D.) of $.035 \pm .050$ hr^{-1} is comparable with the values obtained in the ^{14}C-urea experiments and the t-test indicated no significant difference ($t = .492$). The correlation between the two methods for measuring the urea uptake rate is represented in Figure 3. The slope of the regression does not differ significantly from the expected 1:1 ratio at the 5-percent level, and the two methods show

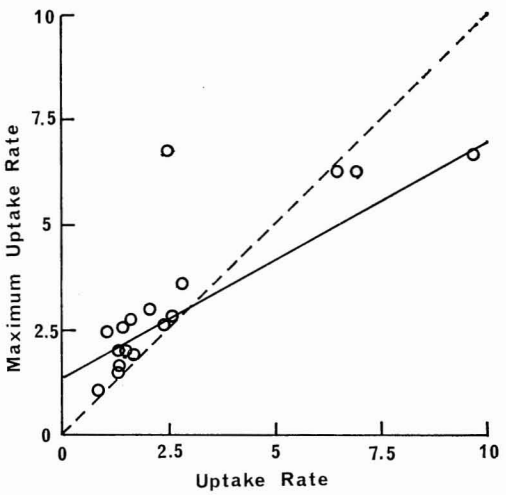

FIGURE 2. The regression of the maximum urea uptake rates, V_m, on the *in situ* urea uptake rates, V, from the radioisotope tracer experiments. Slope of solid line = 0.668; Y-intercept = 0.017; correlation coefficient = 0.81.

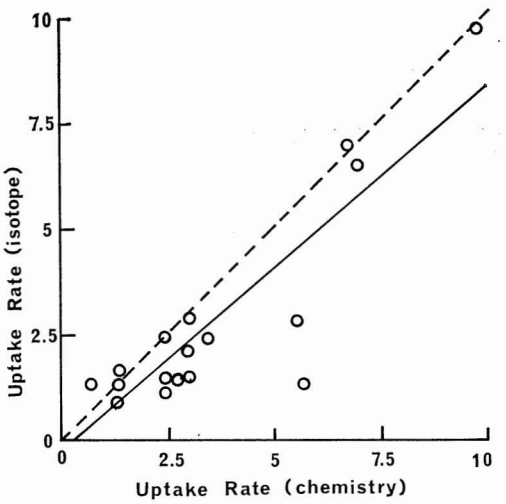

FIGURE 3. The regression of the *in situ* urea uptake rates calculated from time series chemical analyses. Slope of solid line = 0.868; Y-intercept = 0.003; correlation coefficient = 0.881.

relatively good agreement with a correlation coefficient significant at the .1 percent level.

Dark uptake experiments, conducted simultaneously with experiments 17 and 18, indicated that phytoplankton utilization of urea during the dark is approximately 33 per cent of the rate found under daylight conditions, i.e., the dark:light ratio of uptake rates were .010:.030 and .019:.055, respectively. Additional experiments with nitrogen-starved *Monochrysis lutheri* showed a dark:light ratio of .034:.080 or 43 percent.

Uptake of Ammonium

The mean uptake rate ($\pm$ 2 S.D.) of ammonium was .040 $\pm$ .114 hr^{-1}, with a relative standard deviation of 1.399 (Table 4). The uptake rate of ammonium ranged from .003 hr^{-1} to .160 hr^{-1}. Four of the 17 experiments showed a net increase in ammonium, indicating that there was some excretion from the microzooplankton populations in the samples; thus, the observed net uptake rates were somewhat lower than the total uptake rate. The uptake rate of ammonium accounted for 44.8 percent of the total nitrogen uptake rate and ranged from 10.7 to 88.9 percent. The uptake rate reported is based on ambient NH_4^+ concentration being used as the initial concentration value (T_0). The actual T_0 samples were significantly higher than was the ambient concentration and, therefore, have a mean uptake rate of .067 $\pm$ .120 hr^{-1}; however, the ambient ammonium value gave much better agreement with the remaining data points in the time series.

Uptake Rate of Nitrate-Nitrite

In the case of nitrate-nitrite, the mean uptake rate ($\pm$2 S.D.) was .014 $\pm$ .040 hr^{-1}, with a coefficient of variation of 1.455 (Table 4). The nitrate uptake rate provided only 10.1 percent of the total nitrogen uptake rate and ranged from 0.0 to 25.2 percent.

DISCUSSION

The ^{14}C-tracer experiments provided a mean maximum uptake rate (V_m) for urea of .035 $\pm$ .046 hr^{-1} and a mean *in situ* uptake rate, as determined by the time series nutrient analysis, of .035 $\pm$ .050 hr^{-1}. Although the 95-percent confidence limits are wide, these values are comparable to other urea uptake rates that have been reported in the literature. Carpenter, Remsen, and Watson (1972), using similar ^{14}C-

tracer techniques, found that the coastal diatom *Stephanopyxis costata* could assimilate urea at the rate of .019 hr^{-1} in an area where the average urea concentration was 1.3 μg-at N/liter. Using ^{15}N-labeled urea as the primary nitrogen source in two culture experiments, McCarthy and Eppley (1972) measured urea uptake rates of .033 hr^{-1} for cells in a stationary growth phase and .042 hr^{-1} for cells in the logarithmic growth phase. In pure culture experiments, McCarthy (1972*a*) observed a range of maximum (short term) uptake rates for urea from .0076 hr^{-1} for *Thalassiosira pseudonana* (formerly *Cyclotella nana*) (3H) to .0303 hr^{-1} for *Thalassiosira fluviatilis* (T. fluv.), while longer incubations resulted in rates ranging from .0035 hr^{-1} for *Ditylum brightwelli* to .0188 hr^{-1} for *Thalassiosira fluviatilis* (Actin.). The mean urea decomposition rate and cell concentration data provided by Remsen, Carpenter, and Schroeder (1972*a*) and the nitrogen content:cell factor presented in Carpenter, Remsen, and Watson (1972) provide an estimated urea uptake rate of .034 hr^{-1} for natural populations of phytoplankton in the Savannah estuary in which the mean urea concentration was .21 μg-at N/liter. For a single station, located in the vicinity of the Whites Point sewage outfall in California, McCarthy (1972*b*) found a mean urea uptake rate (± 2 S.D.) of .00095 $\pm$ .00141 hr^{-1} and an average urea concentration (± 2 S.D.) of .17 $\pm$ .27 μg-at N/liter. The location was also characterized by high population biomass and carbon productivity.

Our observed rates of urea uptake exhibited a wide range of values. The maximum uptake rates, determined with ^{14}C-labeled urea, ranged from .009 hr^{-1} to .090 hr^{-1}, while the *in situ* rates, from the radioisotope experiments, ranged from .008–.097 hr^{-1}. The uptake rates derived from the time series experiments produced values from .007 hr^{-1} to .098 hr^{-1}. In addition to those factors that have already been shown to be partially responsible for this variability, a number of other variables must also be considered. Guillard (1963) and McCarthy (1972*a*) have illustrated that the ability to utilize urea and the associated rate of uptake varies among species of phytoplankton; therefore, any variation in the species composition can have a marked effect on utilization rate. The preconditioning effects of various forms of fixed nitrogen nutrients on the population must also be considered, along with the synergistic effects of other dissolved constituents.

The uptake rates of ammonium derived from the time series analyses were .040 $\pm$ .114 hr^{-1}, which excluded the high T_0 values, and .067 $\pm$ .120 hr^{-1} which included the high T_0 values. McCarthy and Eppley (1972) showed ammonium uptake rates of .031 hr^{-1} for populations in the stationary growth phase and .032 hr^{-1} for the logarithmic phase of growth. Although these cultures were grown in low-nutrient surface water, the preconditioning nutrient concentrations were not indicated. Caperon and Meyer (1972*b*) found a mean maximum uptake rate (± 2 S.D.) of .607 $\pm$ .697 hr^{-1} for several pure culture populations that had been preconditioned to very low nitrogen concentrations. Off the Whites Point sewage outfall, McCarthy (1972*b*) found a mean ammonium uptake rate (± 2 S.D.) of .00096 $\pm$ .00033 hr^{-1} under conditions of very low ammonium concentration (.17 $\pm$ .12 μg-at N/liter). In these two investigations, the C:N ratios indicate that both populations were nitrogen deficient; therefore, the significant difference in the nutrient uptake rates is the result of the relative level of nutrient input. In the nitrogen-deficient pure culture experiments of Caperon and Meyer (1972*b*), the ambient nutrient concentration within the growth chambers was raised to 2–6 μg-at N/liter and, as a result, the uptake rates measured represent V_m. On the other hand, McCarthy (1972*b*) added 0.1 μg-at N/liter to the already low nutrient concentration, so that the uptake rate that he measured represents the *in situ* rate (V). Under conditions of nutrient saturation, the measured ammonium uptake rate represents the growth rate of a population that is saturated relative to fixed nitrogen. This rate must be on the same order as any other measure of population growth. The order-of-magnitude-higher rates reported by Caperon and Meyer (1972*b*) represent rapid uptake potential of a nutrient-limited population, an uptake potential which cannot be maintained for very long before the population becomes saturated. Our values are comparable to the phytoplankton growth rates and thus support our contention that the growth rate of

Kaneohe Bay phytoplankton is not limited by fixed nitrogen availability. The very much lower values reported by McCarthy (1972*b*) represent either considerable nutrient limitation or difficulties in accurately determining the phytoplankton population size in organic nitrogen units.

The ammonium uptake rates ranged from .003 hr^{-1} to .160 hr^{-1}. Here also variability probably results from a combination of factors, including species composition and interactions with other dissolved constituents.

The utilization rate of nitrate is more difficult to interpret due to the low concentration of nitrate in the environment. The uptake rate derived from the time series experiments had a mean value (± 2 S.D.) of $.013 \pm .040$ hr^{-1} and ranged from 0.0 hr^{-1} to .081 hr^{-1}.

The order of preference, based on the relative rates of nutrient uptake, appears to be ammonium > urea > nitrate, which agrees with the previous investigations of Hattori (1957), Grant, Madgwick, and Dal Pont (1967), and McCarthy and Eppley (1972).

Some of the variability in nutrient uptake rate might be explainable in terms of differences in the size of the phytoplankton, as the work of Eppley, Rogers, and McCarthy (1969) suggests. However, there was no correlation between the fraction of the chlorophyll passing through a 20-μ filter and the nutrient uptake rate. This indicates no size dependence for uptake, although such a conclusion may only apply to a nutrient-saturated environment.

Assuming effluent input concentrations of 23 μg-at/liter for urea, 48 μg-at/liter for nitrate-nitrite, and 1590 μg-at/liter for ammonium (unpublished data) and a sewage input rate of 3.958×10^5 liters/hr (Caperon, Cattell, and Krasnick 1971), we can calculate that the total nitrogen input rate will give 6.575×10^8 μg-at N/hr. The nitrogen input rate of the stream runoff has been estimated by Young, Morphew, and Burbank (1969) to be 6.663×10^7 μg-at N/hr, giving a total nitrogen input ratio of 7.241×10^8 μg-at N/hr. Caperon (1975) calculated the total volume of the southern sector to be 73.6×10^9 liters; therefore, the specific input rate is .010 μg-at N/liter/hr. Using the mean particulate organic nitrogen (3.474 μg-at N/liter) provided by this investigation, we estimated that the sewage effluent and stream runoff contribute approximately .003 hr^{-1} to the total mean nitrogen uptake rate of .086 hr^{-1}. Consequently, these nutrient sources can contribute only 3.5 percent of the total nitrogen uptake rate. Therefore, in the southern sector of Kaneohe Bay, regenerated nitrogen must supply the primary nutrient source to support the large population biomass and high rate of productivity. In terms of specific chemical species, it is apparent from the data presented that ammonium and urea are the most abundant regenerated forms of available nitrogen and provide the primary nitrogen source for growth and speciation.

The total nitrogen uptake rate can be estimated as the sum of the ammonium plus urea plus nitrate. Using the observed mean uptake rates of 0.040, 0.033 and 0.013 hr^{-1} for the respective uptake rates under daylight conditions, and 50, 35, and 0.0 percent of these values for dark uptake rates, we calculated a mean nitrogen uptake rate of 0.062 hr^{-1} for a 12-hour light, 12-hour dark day. This can be compared with carbon uptake data from Lamberson (1974) for the southern sector of Kaneohe Bay. He gave a mean productivity index value of 6.9 mgC (mg Chlorophyll-*a*)$^{-1}$ hr^{-1} for July, August, September, and October 1973. Using the C/Chlorophyll-*a* ratio of 54 given by Caperon, Harvey, and Steinhilper (1976) to convert this rate to inverse time units and assuming zero carbon fixation for 12 hours of the day, we get a carbon uptake rate of 0.064 hr^{-1}. Under steady-state conditions these two rates should be equal. Indeed, given the precision possible with these techniques, the agreement is remarkably good. This supports the contention that the phytoplankton is receiving a saturating supply of fixed nitrogen and that most of this supply is in the form of ammonium and urea.

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